

## Antiviral activity and Antioxidant role of phenolics from *Sophora interrupta* Bedd in NDV induced oxidative stress in chickens

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### Abstract

The present investigation is taken up to evaluate the antiviral efficacy of phenolics isolated from *Sophora interrupta* Bedd and their antioxidant role in the brain and lungs of chicken during Newcastle disease virus (NDV) induced oxidative stress. The activity levels of selected antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) levels were significantly decreased in brain and lung tissues of NDV infected animals over controls causing oxidative stress. In addition, histopathological alterations disclosed that lungs of NDV infected chicken were affected severely as evidenced by the alterations in alveolar cell morphology, congestion, necrotic and degenerative changes whereas degeneration of Purkinje cells, neuronal necrosis, degeneration in myelin sheath and compression of cells were observed in the brain of NDV infected chickens. These reduced antioxidant defence mechanisms and histopathological abnormalities were restored to normal when chicken were pre-treated with the phenolics isolated from *Sophora interrupta* Bedd at the dose of 300 mg/Kg Bw/day for one week. Pre-treatment with the phenolics isolated from the above medicinal plant also caused significant reduction in the titre levels of NDV. These results suggest that pre-treatment with the phenolics isolated from *Sophora interrupta* Bedd exhibited significant antiviral activity and thus the plant extract may be used as a prophylactic treatment for the prevention of NDV infection in chicken.

**Keywords:** Newcastle Disease Virus; *Sophora interrupta* Bedd; Antioxidant enzymes; Brain; Lung; Chicken.

### Introduction

Newcastle disease (ND) is one of the major viral diseases of poultry which causes huge damage than the diseases caused by bacterial and fungal infections. It has been demonstrated that oxidative stress plays a dominant pathogenic role in number of both DNA and RNA viral diseases [1, 2, 3, 4, & 5] and the overwhelming production of ROS induces lipid peroxidation, membrane damage, mitochondrial dysfunction and inflammatory injuries [6].

The oxidative stress can be defined as imbalance between antioxidants and pro-oxidants. Little amount of reactive oxygen species (ROS) such as hydroxyl radicals (HO), superoxide anions (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are constantly generated in aerobic organisms in response to both external and internal stimuli [7]. In normal conditions, a balance is maintained between the formation and removal of these free radicals and the

cells are protected from any damage due to these ROS. Any alteration in the levels of these substances causes oxidative stress which are characterized by 1) loss of glutathione mediated metabolism, 2) Inhibition of protective enzymes such as superoxide dismutase, 3) conformational changes in the biomolecules such as lipids, protein and DNA. In order to protect the cellular damage caused due to copious production of free radicals during different degenerative diseases, cells are protected against oxidative stress by an interacting network of antioxidant enzymes [8&9]. The detoxification pathway is the result of multi-enzyme system with superoxide dismutases, catalases and various peroxidases.

It is well established that the ROS play an important role in many pathological conditions in animal systems during the infection caused by different microorganisms like viruses, bacteria and fungi. The physiologically controlled ROS levels play a key role in the hosts' counter attack to invading microbes [10&11]. Previous studies reported that oxidative stress caused by Paramyxoviruses

and Influenza [12] and Hepatitis B virus, Sendai virus, cytomegalo virus and HIV [13, 14&15] was implicated to the dramatic increase in the levels of ROS.

Although a few nonspecific antiviral drugs are being used to control the NDV infection, continuous usage of these synthetic drugs cause development of resistance in virus. Hence, there is a need to search for new and effective compounds from natural medicinal plants to control the viral infections.

Many Chinese herbal medicines (CHM) or Chinese herbal ingredients (CHI) have been reported to exhibit positive effects on immune enhancement with minimal toxicity and side effects [16&17]. Animals treated with CHM or CHI have also been shown a reduced incidence of infectious diseases and an increased immune response [18].

It has been documented that the active flavonoids of *Sophora flavescens* showed remarkable protection against cellular oxidative damage. Ma et al. (2002) have identified specific active ingredients such as anagyrine, oxymatrine, sophoranol, wogonin and oroxylin extracted from *Sophora flavescens* Ait and *Scutella baicalensis* George which demonstrated potent or moderate antiviral activities against Respiratory syncytial virus (RSV), a paramyxovirus. Ma et al. (2005) evaluated the medicinal value of two plants belonging to CHM, *Legustrum leucidum* and *Schisandra chinensis* and suggested that the diets supplemented with these extracts significantly elevated lymphoblastogenesis of the birds and antibody titer against Newcastle disease Virus (NDV). Guye (1999) has stressed the importance of ethnoveterinary medicine in controlling different poultry diseases using locally available natural products. Earlier studies in our laboratory reported anti-NDV activity of crude extract/compounds isolated from *Sophora interrupta* Bedd using embryonated eggs and cell culture studies. The present work is carried out to evaluate the *in vivo* antiviral activity of Phenolics isolated from *Sophora interrupta* Bedd with particular reference to antioxidant metabolism in lung and brain tissues of chicken during NDV-induced oxidative stress.

## Materials and methods

### Virus

Komorav mesogenic strain of NDV was obtained from the Department of Epidemiology, Sri Venkateswara Veterinary University (SVVU), Tirupati. The viral titre was determined by HA test and the stock were stored in  $-40^{\circ}\text{C}$  for further experiments.

### Virus propagation

The virus titre of NDV was maintained in Embryonated Chicken Egg (ECE) allantoic fluid passages. Infection free one day old ECEs were collected from Poultry division, Sri Venkateswara Veterinary University, Tirupati and were swabbed with 70% alcohol and incubated in humidified egg incubator at  $37^{\circ} \pm 2^{\circ}\text{C}$  for nine

days. The viral stock was diluted with PBS (1:100), pH 7.2-7.4 and then filtered through 0.4 nm membrane filter. 100  $\mu\text{L}$  of filtered virus was inoculated into allantoic cavity of nine day old ECE. NDV infected eggs were incubated at  $37 \pm 2^{\circ}\text{C}$  for four days. Embryonic death during 24h was not considered since the mortality of embryos might have been due to trauma and/or non-specific causes but not due to NDV infection. As soon as the embryonic death was observed, the eggs were chilled in refrigerator at  $4^{\circ}\text{C}$  for 6-24 hours. The allantoic fluid was collected and HA test was performed to determine the titre of the virus and preserved at  $-40^{\circ}\text{C}$ .

### Maintenance of animals

One day old male layer chickens (*Gallus domesticus*; BV300; weighed:  $33 \pm 3\text{g}$ ) were selected as experimental animals for *in vivo* antiviral assay against NDV. The chickens were purchased from Balaji Hatcheries Pvt. Ltd. Chittoor, A.P., India. Animals were maintained in isolators with *ad libitum* access to feed and water in an air conditioned environment ( $35 \pm 2^{\circ}\text{C}$ ) with a 12:12 h light/dark cycle. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India, with the approval of the institutional Animal Ethics Committee (IAEC), Sri Venkateswara University, Tirupati (vide resolution number 59/2012/(i)/a/CPCSEA/IAEC/SVU/WR-CBE dated 8<sup>th</sup> July 2012).

### Extraction of Phenolics

The Phenolics from the roots of *Sophora interrupta* Bedd were extracted according to the method of Singh et al ([2]. 100gm of *Sophora interrupta* Bedd root powder was soaked in 500 mL of Methanol-water (80:20v/v) and subjected to ultrasonication (Branson Sonifier, USA) at 60% duty cycles for 15 min at  $4^{\circ}\text{C}$  followed by centrifugation at 7500 rpm for 15 min. After treatment with charcoal, the extract was distilled and concentrated under reduced pressure in the Buchi rotovapour followed by lyophilisation. The lyophilized powder was used for further studies.

### Determination of Infectious Dose-50 of virus (ID<sub>50</sub>)

Chickens were infected intramuscularly with different dilutions of NDV and the mortality rate for each dose was noted. The median Infectious Dose-50 (ID<sub>50</sub>) was determined by the method of Reed and Muench [23&24].

### Prediction of Maximum non-toxic effect (MNTE) of plant extract

Different concentrations of *Sophora interrupta* Extract (SE) viz; 150, 300, 450, 600, 750 and 900 mg/kg of body weight/day was given to

chicks through intramuscular mode of injection for one week. The maximum non-toxic effect (MNTE) of SE in chickens was determined.

## Experimental design

The chickens were divided into 4 groups, each group consists of 6 chicks and were used for studying the effects of *Sophora interrupta* Bedd root extract.

- Group-I - Chicks served as negative control (only saline)
- Group-II - Chicks served as positive control (NDV-infected)
- Group-III - NDV infected chicks pre-treated with *Sophora* root extract (NDV+SE)
- Group-IV - NDV infected chicks pre-treated with Ribavirin (NDV+RB)

## Isolation of tissues

After stipulated duration (i.e; on 15<sup>th</sup> day), the animals were sacrificed and the lungs and brain tissues were isolated immediately. They were weighed and frozen in liquid nitrogen and were stored at -40°C until analysis. The organs were thawed and used at the time of biochemical analysis.

## Biochemical analysis

Superoxide dismutase activity was determined according to the method of Misra and Fridovich [24]. Catalase activity was measured by a slightly modified version of Aebi and Packer [25]. Glutathione Peroxidase (GPx) activity was determined by the method of Rotruck et al. [26]. Glutathione reductase activity was determined by a slightly modified method of Carlberg and Manervik, [27]. Glutathione-S- transferase activity was measured as per the method of Habig et al. [28].

## Histopathology

Soon after carnage of different experimental chickens, the brain and lung tissues were immediately isolated and fixed in 10% buffered formalin. The tissues were subjected to processing for dehydration and paraffin embedding. Hematoxylin and Eosin (H&E) were used to stain the thin sections (25µm) of brain and lung tissues followed by the examination of histological changes under the light microscope.

## Statistical analysis

The data was analyzed using ANOVA programme to study the differences between means of each experimental group by using Microsoft Excel. The significance was considered at P values less than 0.05 (p<0.05). All the statistical analysis was calculated by using Statistical Program of Social Sciences (SPSS). All values were expressed as mean ± SD (n=6).

## Results

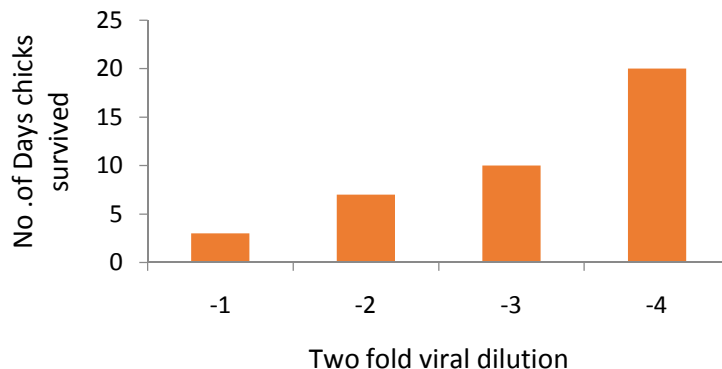
The present study was carried out to determine the anti-NDV and antioxidant effects of *Sophora interrupta* Bedd root extract (SE) in brain and lung tissues of chicken during NDV infection and on pre-treatment with *Sophora* root extract (SE).

## Infective Dose (ID<sub>50</sub>)

Different dilutions of NDV were infected through intramuscular injection to one day old chickens and they were monitored for clinical manifestations (signs and symptoms) and mortality. The mortality rate of chickens was significantly decreased by increasing the dilution of virus. The ID<sub>50</sub> of NDV was calculated as 10<sup>-2.1</sup> (2<sup>-3</sup>) of two fold serial dilution of NDV. Besides ID<sub>50</sub> of NDV, the survival and mortality rate of chickens at different dilutions of NDV was determined. The viral dilution above 2<sup>-4</sup> is inadequate to infect birds and they were survived for more than two weeks. In contrast, the NDV dilution below 2<sup>-3</sup> is sufficient for infection as the chickens die within 11 days. The NDV infected chickens were observed for pathological signs and symptoms like closed eyes, twisted neck, paralysis to legs, moderate decrease in body weights and minimal intake of food and water (Table -1 & Figure: 1).

**Table 1:** Determination of ID<sub>50</sub> of NDV in chickens

S.No.	Log of Virus Dilution	Infected chicks	Uninfected chicks	Cumulative infected chicks (A)	Cumulative un-infected chicks (B)	Ratio of A/(A+B)	Percentage of infection
1	-1	6	0	16	0	16/16	100
2	-2	4	2	10	2	10/12	83.3
<b>3</b>	<b>-3</b>	<b>3</b>	<b>3</b>	<b>6</b>	<b>5</b>	<b>6/11</b>	<b>54.5*</b>
4	-4	2	4	3	9	3/12	25
5	-5	1	5	1	14	1/15	6.7
6	-6	0	6	0	20	0/20	0



**Figure: 1** Mortality of chicks at different dilutions of NDV

**Prediction of Maximum non-toxic effect (MNTE) of plant extract**

The MNTE of plant extract in chickens was predicted as 600 and 5 mg/kg body weight/day for SE and ribavirin respectively (Table – 2).

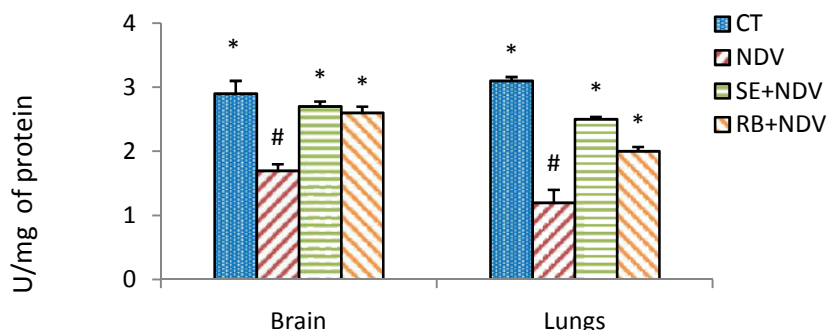
**Table 2:** Determination of Maximum non-toxic effect of SE in chicks

S.No.	mg/kg/body wt.	Mortality rate	% of Survival
1	Saline control	6/6	100
2	150	6/6	100
3	300	6/6	100
4	450	6/6	100
5	<b>600</b>	6/6	<b>100</b>
6	750	5/6	83.3
7	900	5/6	83.3

**Antioxidant enzymes**

The activities of different antioxidant enzymes such as SOD, CAT, GPx, GR and GST levels were assayed in the brain and lung tissues of chicken in control, NDV infected, NDV infected pre-treated with SE and NDV infected treated with Ribavirin (Reference drug). (Figure: 2 to 6).

SOD activity levels were significantly decreased in brain ( $1.7 \pm 0.1$ ) and lung ( $1.2 \pm 0.05$ ) tissues of NDV infected chicken when compared to the control (brain  $2.9 \pm 0.2$ ; lungs  $3.1 \pm 0.06$ ). A Significant increase in SOD of brain ( $2.7 \pm 0.08$ ) and lungs ( $2.5 \pm 0.04$ ) was observed in NDV+SE treated group and NDV+RB group (brain  $2.6 \pm 0.1$ ; lungs  $2.0 \pm 0.07$ ) when compared to NDV infected chickens (brain  $1.7 \pm 0.1$ ; lungs  $1.2 \pm 0.05$ ). No significant changes ( $p > 0.05$ ) were observed between healthy ( $2.9 \pm 0.2$ ) and NDV+SE treated groups of brain tissue ( $2.7 \pm 0.08$ ) (Figure: 2).

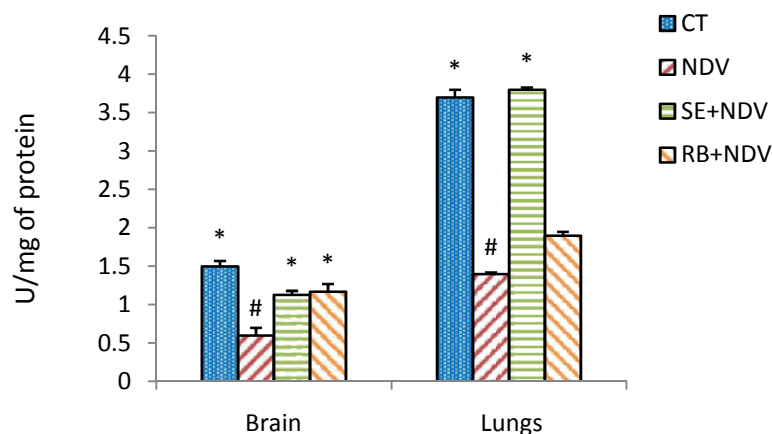


**Figure. 2:** Alterations in the activity levels of Superoxide dismutase (SOD) during NDV infection and on pre-treatment with SESOD

The values are expressed as Mean ± SEM of six individual observations. \*Significant at P<0.05 when compared with NDV (+ve control). #Significant at P<0.05 between control group and NDV infected group.

CAT (U/mg of protein) activity levels were significantly ( $p < 0.05$ ) elevated in NDV + SE group (brain  $1.13 \pm 0.05$ ; lungs  $3.8 \pm 0.03$ ;) when compared to NDV infected positive control (brain  $0.6 \pm 0.1$ ; lungs  $1.4 \pm 0.02$ ). The catalase activity was significantly differ in NDV+RB treated group of brain

( $1.17 \pm 0.1$ ) and non- significant ( $p > 0.05$ ) in case of NDV+RB treated group of lungs ( $1.9 \pm 0.05$ ) when compared to NDV infected group (Figure:3).

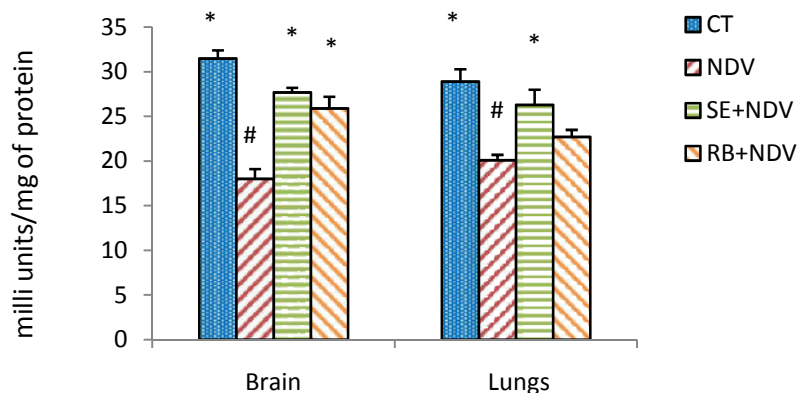


**Figure. 3:** Alterations in the activity levels of Catalase (CAT) during NDV infection and on pre-treatment with SECAT

The values are expressed as Mean  $\pm$  SEM of six individual observations. \*Significant at  $P < 0.05$  when compared with NDV (+ve control). #Significant at  $P < 0.05$  between control group and NDV infected group

Significant decrease was observed in the GPx (milli units/mg of protein) levels in brain ( $18 \pm 1.1$ ) and lungs ( $20.1 \pm 0.6$ ) of NDV infected birds when compared to respective controls (brain  $31.5 \pm 0.9$ ; lungs  $28.9 \pm 1.4$ ). This discrepancy was significantly ( $p < 0.05$ ) recovered by the treatment with SE (brain  $27.7 \pm 0.5$ ; lungs  $26.3 \pm 1.7$ ). Gpx activity in brain was significantly

higher in case of NDV+RB ( $25.9 \pm 1.3$ ) treated groups upon NDV infected group ( $18 \pm 1.1$ ) but, there was no significant change in Gpx activity levels in lungs of NDV infected ( $20.1 \pm 0.6$ ) and NDV +RB ( $22.7 \pm 0.8$ ) groups (Figure: 4).



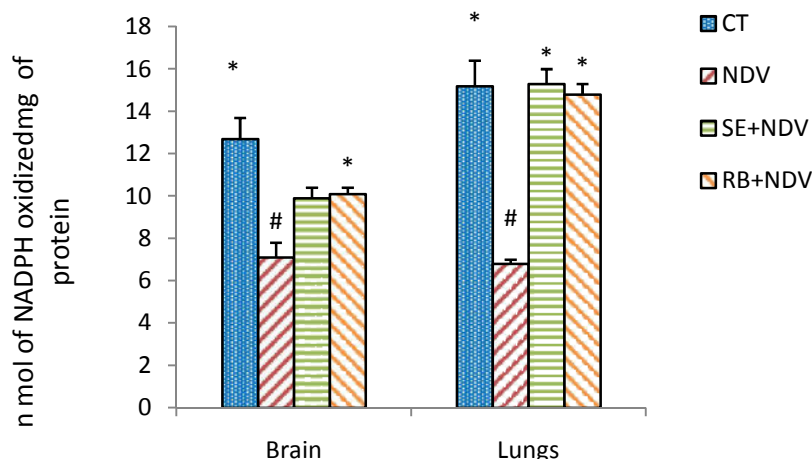
**Figure. 4:** Alterations in the activity levels of Glutathione peroxidase (GPx) during NDV infection and on pre-treatment with SEGPx

The values are expressed as Mean  $\pm$  SEM of six individual observations. \*Significant at  $P < 0.05$  when compared with NDV (+ve control). #Significant at  $P < 0.05$  between control group and NDV infected group

The activity of GR (nmol of NADPH oxidized/mg of protein/min) in brain was moderately elevated in NDV+SE treated group ( $9.9 \pm 0.5$ ) when compared to NDV infected group ( $7.1 \pm 0.7$ ). Significant elevation of GR activity levels was observed in lungs of NDV+SE treated group ( $15.3 \pm 0.7$ )

upon NDV infected group ( $6.8 \pm 0.2$ ). GR activity of NDV+RB (brain  $10.1 \pm 0.3$ ; lungs  $14.8 \pm 0.5$ ) was slightly higher than NDV infected group (brain  $7.1 \pm 0.7$ ; lungs  $6.8 \pm 0.2$ ) (Figure: 5).

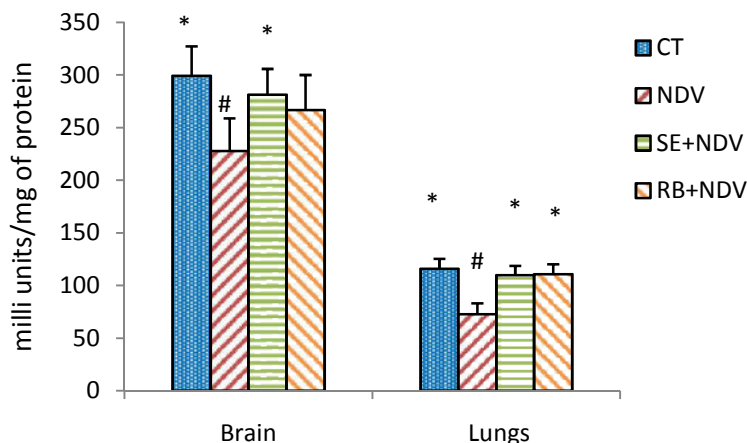




**Figure. 5:** Alterations in the activity levels of Glutathione reductase (GR) during NDV infection and on pre-treatment with SEGR. The values are expressed as Mean  $\pm$  SEM of six individual observations. \*Significant at  $P < 0.05$  when compared with NDV (+ve control). #Significant at  $P < 0.05$  between control group and NDV infected group.

GST (nmol of CDNB conjugate formed/mg of protein/min) activity was significantly ( $p < 0.05$ ) diminished in NDV infected group (brain  $228 \pm 31.1$ ; lungs  $73 \pm 10.1$ ) when compared to healthy subjects (brain  $299.7 \pm 27.7$ ; lungs  $116 \pm 9.4$ ). This deviation was significantly reduced in NDV+SE

group (brain  $281.5 \pm 24.5$ ; lungs  $110 \pm 8.8$ ). GST levels in NDV+RB treated group was moderately increased in brain ( $267 \pm 33.3$ ) and significantly increased in lungs ( $111 \pm 9.2$ ) (Figure: 6).



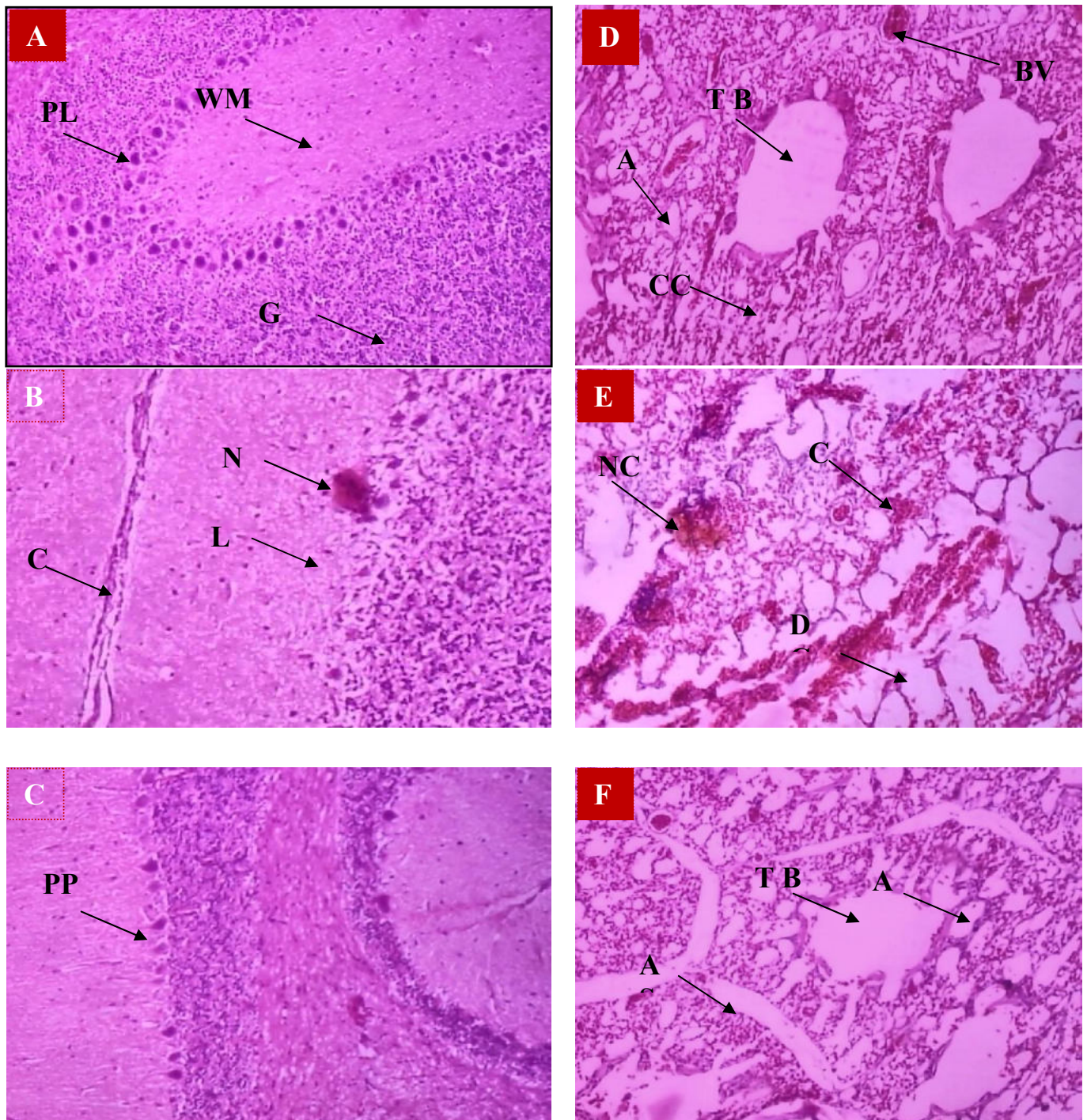
**Figure. 6:** Alterations in the activity levels of Glutathione-s-transferase (GST) during NDV infection and on pre-treatment with SEGST. The values are expressed as Mean  $\pm$  SEM of six individual observations. \*Significant at  $P < 0.05$  when compared with NDV (+ve control). #Significant at  $P < 0.05$  between control group and NDV infected group.

### Histopathological alterations

The histopathological studies revealed that the brain and lung tissues were affected during NDV infection. But the former one affected drastically and moderately the latter. Brain tissue of NDV infected chickens elucidates the deterioration of Purkinje cells,

moderate glial cell population and mild neuronal necrotic changes (Figure 7: B). Congestion and necrosis were observed in the lungs of NDV infected chickens (Figure 7: E). However, these lesions were recovered to normal in NDV+ SE treated chickens (Figure 7: C&F).





**Figure 7:** H&E stained Photomicrographs of (I) Brain of A: negative control; B: NDV-infected (mild neuronal necrosis and increased glial cell); C: NDV+SE (cellular protection). (II) Lungs of D: negative control; E: NDV-infected (deterioration of Clara cells); F: NDV+SE (cellular recovery). **WM:** White matter; **PL:** Purkinje layer; **GL:** Granular layer; **NC:** Necrotic changes; **LP:** Loss of Purkinje layer; **C:** Congestion; **PP:** Protection of Purkinje layer; **BV:** Blood vessel; **TB:** Terminal Bronchiole; **A:** Alveoli; **CC:** Clara cells; **DC:** Degenerative changes; **AS:** Alveolus.





## Discussion

Viruses exert various mechanisms in order to infect and invade the host systems. In addition to the specific pathogenic events, NDV infection causes severe oxidative stress by altering pro/antioxidant status of brain and liver of chicken (Venkata subbaiah et al., 2011, 2013 and 2015). Although a few reports on NDV induced oxidative stress is documented, little attention has been focussed on the ameliorative effects of medicinal plants and their extracts on the oxidative damage caused by NDV. Hence, the present investigation is mainly focussed to study the *in vivo* effect of NDV infection, and the protective effect of *Sophora interrupta* Bedd plant extract with particular reference to antioxidant metabolism.

In order to carry out these studies, standardization for the determination of Median infective dose (ID<sub>50</sub>) for NDV virus was performed using mortality studies on chicken. The chickens were infected intra muscularly (IM) with different dilutions of ND Virus and 50% mortality rate was determined at 2<sup>-3</sup> viral dilution and ID<sub>50</sub> of NDV was calculated as 10<sup>-2.11</sup> and hence, this viral concentration was used in the entire study during NDV infection.

Similarly Maximum Non-Toxic Concentration (MNTC) was also determined for the plant extract (SE) in order to prescribe the dose at which no toxicity is being caused by the plant extract. Prior to evaluating the *in vivo* antioxidant effect of *Sophora* Extract (SE), the toxicity of SE on chickens was determined by intramuscular administration of different concentrations of SE. The MNTC for SE was found to be 600mg/kg/body Wt. Hence, the experiments were carried out in such a way that the treatment with SE concentration did not exceed 400mg/kg/body wt. during NDV infection.

Although various studies revealed pathophysiological effects of NDV infection and its treatment in chickens, very little information is available on the effect of NDV induced oxidative stress. Recent studies from our laboratory reported that mesogenic strain of NDV induces oxidative stress and is also one of the possible mechanisms of NDV infection through which it interferes with pro and antioxidant status at the tissue level (brain and liver) in chickens and vitamin-E mitigates NDV infection in chickens (Venkata Subbaiah et al., 2011). This can be concurred with the obtained results that the antioxidant levels in brain and lungs were drastically lessen in NDV infected group upon healthy controls.

Earlier studies in our laboratory revealed that NDV infection in chicken affects vital organs such as brain, liver and heart by inducing oxidative stress as evidenced by an increase in lipid peroxidation and alterations in the antioxidant metabolism. It is well documented that the elevated lipid peroxidation causes significant depletion in the activity levels of antioxidant enzymes such as SOD, CAT and glutathione based enzymes. It is also well established that SOD is considered as a first line of antioxidant defence mechanism which converts Reactive Oxygen Species, the superoxide into hydrogen peroxide and molecular oxygen through CAT and GPx. In the present study, SOD and CAT activity levels were significantly decreased in brain and lung tissues of NDV-infected chicken when

compared to control animals leading to accumulation of superoxides and hydrogen peroxide causing oxidative stress.

Similarly the glutathione dependant enzymes such as GST, GPx and GR which are primarily involved in detoxification mechanisms were found to be significantly reduced in brain and lung tissues of NDV-infected chicken. The reduced activity levels of these enzymes suggest improper scavenging activity of free radicals during viral infection. The decreased activity levels of glutathione-dependant enzymes might also be implicated to the alterations in glutathione metabolism as evidenced by reduced GSH levels and alteration in the GSH/GSSG ratio which is considered to be the most crucial redox couple that regulates intrinsic antioxidant defence system (Venkata subbaiah et al., 2011).

In the present study, the changes in the glutathione metabolism of NDV infected chickens were restored by the administration of SE. Pre-treatment with SE has increased the activity levels of glutathione-dependant enzymes in brain and lungs of chickens during NDV infection. Usually, GSH concentration is directly proportional to the activity levels of Glutathione dependant enzymes. The viral-induced alterations in glutathione metabolism was restrained by the treatment with SE and this antioxidant effect might be due to protection of GSH from oxidative stress in the brain and lungs of NDV-infected chickens.

Consistent with our results, the findings of Yashoda et al., [29] reported that Respiratory Syncytial Virus (RSV) induces oxidative stress by modulating the expression of antioxidant enzymes and causes oxidative injury in lungs. Similarly it is reported that the activities of SOD, CAT, GPx, GR, GST and levels of GSH were decreased in brain and liver of NDV infected chickens over controls (Venkata Subbaiah et al., 2011).

The reduced mortality rates of NDV-infected chickens during pre-treatment with SE demonstrated the protective role of SE. Waihenya et al. [30] evaluated the efficacy of the crude extract of *Aloe secundiflora* in chickens experimentally infected with NDV and concluded that reduced mortality rate and the severity of clinical signs during the acute phase of infection in *Aloe* treated chickens compared with non-treated ones. *Aloe vera* and *Aloe spicata* were used to treat NDV infected chickens in Zimbabwe [31].

Acemannan, a water soluble polysaccharide, has been proved to have a direct antiviral effects against a number of enveloped viruses including NDV by inhibiting their multiplication [32]. One of the Chinese Herbal Medicines (CHI), Epimedium flavone, was reported to be more potent in promoting the humoral immune response to NDV infection *in vivo* and acts as immune stimulators for chickens [33]. Wegner et al. [34] demonstrated that the plant derived polyphenols have shown marked anti-influenza virus effect. Village fowl farmers in Botswana have reported that feeding the fowl on green mulberry leaves to induce diarrhoea before the NDV infection and claim that fowl that have been subjected to this treatment do not contact the disease [35].

Silymarin, an extract from the *Silybum marianum* (milk thistle) plant containing various flavonolignans, possesses free radical scavenging activity and prevents free radical formation by inhibiting



specific ROS-producing enzymes, or improves the integrity of mitochondria in stress conditions. In addition to that, it is reported to maintain an optimal redox balance in the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants [36].

It is well established that antioxidants have many potential applications, especially in relation to human and animal health, both in terms of prevention of disease and therapy. Nadia *et al.* [37] found that oily extracts of Onion and Garlic have virucidal action on different strains of Newcastle disease virus. Hegazi *et al.* [38&39] reported that the addition of *Propolis* to NDV induces a significant reduction of infectivity. Methanolic extract of the aerial part of *S. argel* plant and the extracts of four indigenous plants viz; *Aglaia elaeagnoides*, *Zingiber capitatum*, *Cassia fistula* and *Acacia raddiana* have shown demonstrable antiviral activity upon Newcastle disease virus.

The antiviral activity was attributed to the occurrence of some inhibitory substances in the plant extracts which resulted in complete inhibition and/or reduction in virus production [40]. As the free radicals were shown to be one of the consequences of NDV infection, antioxidants from natural medicinal plants have been experimentally proved as effective protective agents against viral induced oxidative stress. Hence, the antioxidants not only have direct role on scavenging the free radicals but also has an indirect effect on viral inhibition.

## References

- [1]. Peterhans E. Sendai virus stimulates chemiluminescence in mouse spleen cells. *Bioch. Biophys. Res. Comm.* 1979; 91: 383–392.
- [2]. Peterhans E, Grob M, Burge T and Zanoni R. Virus-induced formation of reactive oxygen intermediates in phagocytic cells. *Free Radical Res Comm.* 1987; 3:39-46.
- [3]. Muller. Reactive oxygen intermediates and Human Immunodeficiency Virus (HIV) infection. *Free. Radi. Biol. Med.* 1992; 13: 651–657.
- [4]. Pasquier R, Olivier Y, Auclair C and Packer L. Oxidative Stress, Cell Activation and Viral Infection. 1994; (Conference).
- [5]. Ravindra BK, Koteswar RV, Nanda KY, Kishore P, Subbaiah KCV, Bhaskar M, Lokanatha V and Nagaraju C. Identification of substituted [3, 2-a] pyrimidines as selective antiviral agents: 3 Molecular modeling studies. *Antiviral. Res.* 2012; 95: 118–127.
- [6]. Akaike T Role of free radicals in viral pathogenesis and mutation. *Rev. Med. Virol.* 2001; 11:87-101.
- [7]. Lee Y, Galoforo SS, Berns CM, Chen JC, Davis BH, Sim JE, Corry PM and Spitz DR. Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J Biol Chem.* 1998; 273: 5294-5299.
- [8]. Sies H, Sharov VS, Klotz L and Briviba K. Glutathione peroxidase protects against peroxynitrite-mediated oxidations. A new function for seleno proteins as peroxynitrite reductase. *J Biol Chem.* 1997;272: 27812-27817.
- [9]. Davies K Oxidative stress: The paradox of aerobic life. *Bio chem Soc Symp.* 1995; 61: 1-31.
- [10]. Hennet T, Peterhans E and Stocker R Alterations in antioxidant defences in lung and liver of mice infected with Influenza A virus. *J Gen Virol.* 1992; 73: 39-46.
- [11]. Benzer F and Yilmaz S Effects on oxidative stress and antioxidant enzyme activities of experimentally induced *Ornithobacterium rhinotracheale* infection in broilers. *J Ani Vet Adv.* 2009; 8 (3): 548-553.
- [12]. Peterhans E, Grob M, Burge T and Zanoni R. Virus-induced formation of reactive oxygen intermediates in phagocytic cells. *Free Radical Res Comm.* 1987; 3:39-46.
- [13]. Akaike M oxygen free radicals as pathogenic molecules in viral diseases. *Proc. Soc. Expt. Biol. Med.* 1991;198: 721-729.
- [14]. Ikeda T, Shimokata K and Daikoku T. Pathogenesis of cytomegalovirus-associated Pneumonitis in ICR mice: possible involvement of superoxide radicals. *Arch Virol.* 1992; 127: 11-24.

## Conclusion

The results of the current study revealed that the NDV infection causes increased oxidative stress which might be implicated to the depletion of antioxidant metabolism. This incongruity was normalized by the treatment to NDV challenged chickens with *Sophora interrupta* Bedd root extract (SE). In addition to this, histopathological studies demonstrated that the Phenolics of *Sophora interrupta* Bedd promised to protect the tissues from NDV infection. Thus, the antioxidant activity of *Sophora interrupta* Bedd indirectly caused reduction of viral infection. This clearly supports the protective role of SE as an antioxidant therapy to neutralize the ill effects of oxidative stress in NDV infected chickens.

## Conflict of interest

The authors declare that they have no conflict of interest.

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- [15]. Schwarz B. Oxidative stress during viral infection: A review. *Free. Radical. Biol. Med.* 1996; 21:641-649.
- [16]. Liang R, Guo KK, Yi L, Zhang YX, Li JQ and Yue ZQ. Study of Chinese herbal medicinal immune stimulators on immune enhancement in chickens. *Chin J Vet Sci Technol.* 1998; 28: 11-13.
- [17]. Liu JG, Hu YL, Chen YK, Zhang BK and Kong XF. The safe concentrations of several components in natural drugs on CEF. *Prog Vet Med.* 2002; 23: 88-91.
- [18]. Hu YL. Progress in the study of immunopharmacology of Chinese herbal medicine. *Chi. J Immunol.* 1997;13: 96-98.
- [19]. Ma SC, Du J, But PP, Deng XL, Zhang YW, Ooi VE, Xu HX, Lee SH and Lee SF Antiviral Chinese medicinal herbs against Respiratory Syncytial Virus. *J Ethnopharma.* 2002; 79 (2): 205-211.
- [20]. Ma D, Shan A, Chen Z, Du J, Song K, Li J and Xu Q. Effect of *Ligustrum lucidum* and *Schisandra chinensis* on the egg production, antioxidant status and immunity of laying hens during heat stress. *Arch Anim Nut.* 2005; 59 (6): 439-47.
- [21]. Guye FHE. Ethno-veterinary medicine against poultry diseases in African villages. *World Poul Sci J.* 1999; 55: 187-198.
- [22]. Singh UP, Sarma BK, Singh DP and Bahadur A Plant growth-promoting Rhizobacteria-mediated induction of Phenolics in Pea (*Pisum sativum*) after infection with *Erysiphe pisi*. *Current Microbiology.* 2002; 44, 396-400.
- [23]. Reed, LJ and Muench H A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* 1938; 27: 493-497.
- [24]. Misra HP and Fridovich I The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol Chem.* 1972; 247:3170-3175.
- [25]. Aebi H and Packer L Catalase *in vitro*. *Methods in Enzymol.* 1984; 105: 121-126.
- [26]. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG Selenium: Biochemical roles as a component of glutathione peroxidase. *Science.* 1973; 19:588-590.
- [27]. Carlberg I and Mannervik B Glutathione reduction. In: *Methods in Enzymology.* Academic Press in Orlando. 1985; 113: 484-499.
- [28]. Habig WH, Pabst MJ and Jakoby WB Glutathione-s-transferases. *J. Biol, Chem.* 1974; 249: 7130-7139.
- [29]. Yashoda M, Tianshung L, Castro SM, Garofalo RP and Casola A Respiratory syncytial virus induces oxidative stress by modulating antioxidant enzymes. *Am J Respr Cell Mol.* 2009; 41: 348-357.
- [30]. Waihenya RK, Mtambo MMA, Nkwengulila G and Minga UM Efficacy of the crude extract of *Aloe secundiflora* against *Salmonella gallinarum* in experimentally infected free range chickens in Tanzania. *J of Ethnopharmacol.* 2002; 79 (3): 317-323.
- [31]. Marizvikuru M, Evison B, Michael C and Tinyiko EH. Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. *Int J Appl Res Vet Med.* 2005; 3(2): 2005.
- [32]. Kemp MC, Kahlon JB, Chinnah AD, Carpenter RH, McAnalley BH, McDaniel HK and Shannon WM. *In vitro* evaluation and the antiviral effects of acemannan on the replication and pathogenesis of HIV-1 and other enveloped viruses: modification of the processing of glycoprotein precursors. *Antiviral Res.* 1990; 13: 83.
- [33]. Kong XF, Hu YL, Yin YL, Wu GY, Rui R, Wang DY and Yang CB Chinese herbal ingredients are effective immune stimulators for chickens infected with the Newcastle disease virus. *Poul Sci.* 2006; 85: 2169-2175.
- [34]. Wegner U, Mundt S, Kliefoth C, Meyer B, Lindequist U and Mentel R Antiviral activity of plant polyphenols against Influenza virus. *Hyg Med.* 2000; 25(3): 75-78.
- [35]. Moreki JC. Small-scale poultry production systems in Serowe-Palapye Subdistrict (Botswana). M.Sc. Thesis, University of Melbourne, Melbourne (1997) Australia.
- [36]. Peter FS. Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives. *Antioxidants.* 2015; 4: 204-247.
- [37]. Nadia MH, El-Sabbagh AH, El-Assily S, Khashaba E, El-Bardini F, El-Ebiary AE and Taha MM. Virucidal effect of Garlic and Onion oily extracts on different strains of Newcastle disease virus. *Ben Vet Med Res.* 1991; 1(1): 205-214.
- [38]. Hegazi AG, El-Berdiny F, El-Assily S, Khashaba E, Hassan N and Popov S. Studies of some aspects of antiviral activity-Influence of *Propolis* on Newcastle disease virus. *Vet. Med. J. Giza.* 1993; 41(2): 53-56.
- [39]. Hegazi AG, El-Berdiny F, El-Assily S, Khashaba S and Abd-El-Hady F Studies of some aspects of antiviral activity-Influence of *Solenostemma argle* on Newcastle disease virus. *Vet. Med. J. Giza.* 1994; 54(5): 435-441.
- [40]. Singh RK, Singh DP and Pathak RC. Antiviral activity of indigenous plants. *Indian J Vet Res.* 1994; 3(2): 10-19.
- [41]. Subbaiah KCV, Raniprameela D, Visweswari G, Rajendra W and Lokanatha V. Perturbations in the antioxidant metabolism during Newcastle disease virus (NDV) infection in chicken-Protective role of vitamin E. *Naturwissenschaften.* 2011; 98: 1019-1026.